In vitro Evaluation of *Phalaris minor* Seeds as Livestock Feed

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ABSTRACT : The nutritional worth of *Phalaris minor* seeds was assessed in comparison to conventional cereal grains like maize and wheat. *P. minor* seeds had higher total ash and cell wall constituents as compared to wheat and maize grains. The CP content of *P. minor* was comparable to wheat grains but higher than maize grains. The *in vitro* studies revealed that the net gas production and availability of ME from *P. minor* was comparable to that of maize but the digestibility of nutrients was significantly ($p<0.05$) lower than that of conventional cereal grains. The digestion kinetic parameters for DM and CP revealed that *P. minor* had the highest ($p<0.05$) soluble fraction (a) followed by wheat and maize. Reverse trend was observed for insoluble but potentially degradable fraction (b). The effective and true DM and CP degradability was significantly ($p<0.05$) higher in wheat grains followed by that in *P. minor* and maize grains. The digestibility of OM and NDF was not affected by replacing cereal grains in concentrate mixture with *P. minor* seeds up to 75 per cent level. But the availability of ME from concentrate mixtures was comparable to control only up to 50% level of replacement. Replacement of cereal grains with *P. minor* did not affect the rapidly soluble fraction and insoluble but potentially degradable fraction of concentrate mixture containing *P. minor* up to 75 per cent, but it was depressed significantly at 100% replacement level. The effective and true degradability of DM of concentrate mixtures containing *P. minor* from 50 to 100 per cent was comparable to that of conventional concentrate mixture (CCM). The wheat based concentrate mixtures showed higher net gas production (208 vs. 201 ml/g DM/24 h), digestibility of nutrients and ME availability (9.64 vs. 9.54 MJ/kg DM) as compared to maize based concentrate mixture. The wheat based concentrate mixture had significantly ($p<0.05$) higher rumen undegradable fraction and effective degradability. The data conclusively revealed that conventional cereal grains could be replaced with *P.minor* seeds up to 75 per cent without affecting the availability of nutrients. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 3 : 363-367)***

Key Words : *Phalaris minor*, *In vitro* Gas Production, *In sacco* Evaluation, Nutrient Availability

INTRODUCTION

Phalaris minor (family, *graminae*) is a fast spreading weed in wheat fields, especially in the Northern States of India, where rice-wheat rotation is predominant. Each plant produces about 300-400, shiny black, very small, flat seeds, which usually contaminate wheat grains. Wheat being the staple food throughout the world, no compromise with it's yield or quality can be made, therefore, it becomes imperative for the scientists to check the propagation of *Phalaris minor* or to find an alternate use of this weed. Different methods have been employed to control weed, but none could eliminate the weed completely. Since it emerges with the germination of wheat seedlings and resembles very closely, making it difficult to recognize in the initial stage of growth. The *P. minor* has many medicinal properties viz. acts as carminative, flushes out the worms and above all it improves the immunity of the animals (Oudhia, 2003). However, information about its nutritive value is lacking. The present study was, therefore, undertaken to assess the nutritive value of *Phalaris minor,* for ruminants.

MATERIALS AND METHODS

In the 1st trial nutritional value of *Phalaris minor* seeds was assessed in comparison to conventional energy supplements like wheat and maize. In the 2nd trial cereal based conventional concentrate mixture (CCM) containing either wheat or maize 30, mustard cake 15, de-oiled mustard cake 15, rice bran 15, de-oiled rice bran 5, wheat bran 10, molasses 7, mineral mixture 2 and common salt 1 par each was prepared. The cereal grains in the concentrate mixture were replaced with *P. minor* seeds on weight basis, at 25, 50, 75 and 100%. The nutritive value of *P. minor* seeds and the concentrate mixtures was assessed by *in-vitro* gas production technique (Menke et al., 1979) and *in sacco* method (Mehrez and Orskov, 1977).

In vitro gas production studies

About 375 mg of the substrate was incubated at 39°C for 24 h in triplicate in 100 ml calibrated glass syringes (procured from M/s Haberle Labortechnik, Germany) containing feedstuff and a buffered rumen fluid. Blank, sample of standard hay and standard concentrate (procured from International Atomic Energy Agency, Vienna) were run in triplicate with each set. Rumen contents were collected from 3 buffalo calves maintained on 2.0 kg conventional concentrate mixture (maize 25, maize oil cake 10, de-oiled mustard cake 20, rice bran 15, de-oiled rice bran 15, wheat bran 12, mineral mixture 2 and common salt 1 part each), 4.0 kg green fodder and 6.0 kg wheat straw. The rumen contents were collected at 0 hr in a thermos flask flushed with CO_2 and maintained at 39°C. The rumen

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contents were blended for 2-3 minutes in blender maintained at 39°C and then strained through 4-layered muslin cloth. The solution, containing 960 ml distilled water, 0.16 ml micro-mineral solution (13.2 g $CaCl_2$ $2H_2O$, 10.2 g $MnCl_2$ $4H_2O$, 1.0 g $CoCl_2$ $6H_2O$ and 8.0 g $FeCl_3$ $6H_2O$ per 100 ml distilled water), 660 ml bicarbonate buffer (35 g $NaHCO_3$ and 4.0 g NH_4HCO_3 per 1,000 ml distilled water), 330 ml macro-mineral solution (5.7 g Na_2HPO_4, 6.2 g KH_2PO_4, 0.6 g $MgSO_4$ $7H_2O$ per 1,000 ml distilled water) and 1.6 ml Resazurine (0.1%), was mixed in a Woulff flask (3 l cap) mixed with magnetic stirrer in a water bath at 39°C, followed by addition of freshly prepared reducing (373.0 mg Na_2S H_2O, 2.6 ml of 1 N NaOH in 62.0 ml distilled H_2O) solution and flushing of CO_2 through a submerged tube. The light bluish solution first turned pinkish then became colourless. Then strained rumen liquor (SRL) was added to the buffer media in 1:2 ratio. The flushing of CO_2 was continued till the last syringe was filled. The tube on the capillary attachment to the syringe was firmly fixed on to the top dispenser bottle. 30 ml (SRL: buffer) solution was pumped in each syringe. The contents in the syringe were mixed by gentle shaking. Air bubbles were brought to the surface and removed through the capillary by careful upward movement of the piston. The clip was closed immediately and exact volume of contents in the syringe was noted and kept in a water bath maintained at 39°C. The contents in all the syringes were swirled at 60 minutes interval for first few hours of incubation so that material does not stick to the walls of the syringe or bottom of the plunger. If at 8 h after incubation the volume of the gas exceeded 70 ml, the actual volume of the gas produced was recorded and then the gas was taken out. After 24 h, volume of gas produced in each syringe was recorded and the contents of syringes were transferred to spout-less beaker, boiled with neutral detergent solution (which dissolves microbial biomass) for assessing the true organic matter (OM) and neutral detergent fiber (NDF) digestibility. The metabolisable energy (ME) was worked out by using the equation developed by Menke and Steingass (1988).

$$\text{ME (MJ/kg DM)} = 0.04+0.1639\ \text{GP}+0.0079\ \text{XP} +0.0239\ \text{XL} \qquad R^2= 0.92$$

Where GP is net gas production in ml/200 mg substrate DM/24 h.

XP is crude protein in g/kg DM

XL is ether extract in g/kg DM

In sacco degradability

For determination of *in sacco* degradability and kinetic parameters, three buffalo calves fitted with permanent rumen fistulae were selected and maintained on 2 kg concentrate mixture (maize 25, maize oil cake 10, de-oiled mustard cake 20, rice bran 15, de-oiled rice bran 15, wheat bran 12, mineral mixture 2 and common salt 1 part each) supplemented with 4 kg green fodder and 6 kg wheat straw. The nylon bags of 8×17 cm stitched with monofilament polyester thread with pore size of 50±10 µ were used. Five grams sample was placed in the nylon bags, which were incubated in the rumen for 2, 4, 6, 8, 10, 12, 24, 36 and 48 h in triplicate. The 'zero h' bags were not incubated in the rumen but were washed in the same manner as incubated bags. After the stipulated period, bags were taken out, washed under tap water, until rinsing water from the bag became colorless. The bags were dried in a forced air oven maintained at 60°C for 48 h. The disappearance of dry matter (DM) was measured as the loss in weight of the bag contents. The residue was analyzed for DM, crude protein (CP) and NDF content. The different physical constants characterizing extent and rate of ruminal degradation, i.e. rapidly soluble fraction (a), insoluble but potentially degradable fraction (b), degradation rate (c) and effective degradability (ED) were calculated according to McDonald (1981). The rumen fill was calculated according to Van Eys (1982). The DM intake was predicted by using the equation of Orskov et al. (1988).

Chemical analysis

Samples of *Phalaris minor* seeds, cereal grains (wheat and maize) and concentrate mixtures were ground to pass through 1 mm sieve and analyzed in duplicate for nitrogen, ether extract and total ash (AOAC, 1995), cellulose (Crampton and Maynard, 1938) and other cell wall constituents (Robertson and Van Soest, 1981).

Statistical analysis

The data of the 1st trial were analyzed by completely randomized design and that of the 2nd trial by 2×5 factorial design (Snedecor and Cochran, 1994) using STATGRAPHICS version 5.0. The means were compared for statistical significance by using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition of *Phalaris minor* seeds in comparison to conventional cereal grains

In order to meet the nutritional requirements of livestock, as well as for manufacturing compounded feed, precise knowledge of feedstuff composition is a prerequisite. The *P. minor* seeds being unconventional feed resource have never been evaluated for their nutritional worth. The *P. minor* seeds had higher total ash, NDF, acid detergent fiber (ADF), cellulose and lignin content and considerably low OM content than that of wheat and maize grains. The CP content of *P. minor* seeds and that of wheat grains was

Table 1. Chemical composition of *Phalaris minor* seeds (% DM basis)

Constituent	*Phalaris minor*	*Zea mays*	*Triticum aestivum*
Total ash	10.0	1.8	1.8
OM	90.0	98.2	98.2
CP	10.9	8.2	11.2
NDF	50.0	46.0	35.0
ADF	18.0	3.5	4.0
Hemi-cellulose	32.0	42.5	31.0
Cellulose	6.0	4.0	2.0
ADL	8.5	-	1.0

Table 2. *In vitro* evaluation of *Phalaris minor* seeds

Parameter	*Phalaris minor*	*Zea mays*	*Triticum aestivum*	Pooled SE
NGP (ml/g DM/24 h)	214.6^{a}	223.3^{a}	320.7^{b}	3.69
NDFD (%)	63.4^{a}	74.6^{b}	84.5^{c}	2.38
TOMD (%)	83.1^{a}	91.9^{b}	95.4^{c}	0.93
ME (MJ/kg DM)	8.9^{a}	9.5^{a}	12.0^{b}	0.36

NGP: Net gas production; NDFD: Neutral detergent fiber digestibility; TOMD: True organic matter digestibility; ME: Metabolisable energy.
Figures with different superscripts in a row differ significantly ($p<0.05$).

higher than that in maize grains. The hemi-cellulose content in *P. minor* and that of wheat grains was considerably lower than that in maize grains (Table 1).

In vitro/in sacco evaluation

The net gas production from *P. minor* was lower ($p<0.05$) than that from wheat but equivalent to that produced from maize grains (Table 2). Amongst the seeds/grains tested, the *P. minor* seeds had the lowest ($p<0.05$) digestibility of NDF and OM. The higher digestibility of nutrients in wheat resulted in higher ($p<0.05$) availability of metabolizable energy (ME) as compared to other grains. The availability of ME from *P. minor* seeds was similar to that of maize.

The digestion kinetic parameters for DM, assessed by nylon bag technique, revealed that *P. minor* had the highest ($p<0.05$) soluble fraction (a) followed by wheat and maize (Table 3). Reverse trend was observed for insoluble but potentially degradable fraction (b). The amount of potentially degradable fraction in wheat and maize was comparable, but the degradation rate (c) was higher ($p<0.05$) for wheat followed by that for *P. minor* and maize grains. The *P. minor* seeds had higher ($p<0.05$) rumen undegradable fraction (UDF) as compared to conventional cereal grains (wheat and maize). The effective and true degradability of *P. minor* was higher ($p<0.05$) than that of maize grains, but lower ($p<0.05$) than that of wheat grains.

Table 3. Digestion kinetic parameters for DM and CP of *Phalaris minor* seeds

Parameter	*Phalaris minor*	*Zea mays*	*Triticum aestivum*	Pooled SE
Dry matter (%)				
a	71.6^{b}	23.9^{a}	36.4^{a}	4.52
b	12.2^{a}	69.9^{b}	60.5^{b}	5.10
c (h^{-1})	0.10^{a}	0.08^{a}	0.14^{b}	0.01
UDF	16.3^{b}	6.2^{a}	3.1^{a}	1.07
ED	81.3^{b}	77.2^{a}	88.1^{c}	0.75
TD	80.2^{b}	76.0^{a}	85.1^{c}	1.08
Crude protein (%)				
a	74.6^{c}	10.4^{a}	50.5^{b}	3.84
b	20.5^{a}	85.4^{c}	47.8^{b}	4.34
c (h^{-1})	0.05	0.09	0.15	0.01
UDF	4.9^{b}	4.2^{ab}	1.8^{a}	0.71
ED	88.6^{b}	76.8^{a}	91.5^{c}	0.49
TD	68.0^{a}	77.6^{b}	85.6^{c}	0.93

a: Rapidly soluble fraction; b: Insoluble but potentially degradable fraction; c: Degradation rate; ED: Effective degradability; UDF: Undegradable fraction; TD: True degradability.
Figures with different superscripts in a row differ significantly ($p<0.05$).

The digestion kinetic parameters for CP revealed that *P. minor* had the highest ($p<0.05$) rapidly soluble fraction followed by that in wheat and maize (Table 3). But, the potentially degradable fraction followed the reverse trend. The potentially degradable fraction in *P. minor* was 25 and 50% less than that in wheat and maize grains, respectively and was degraded at the lowest rate. The minimum potentially degradable fraction with slowest degradation rate resulted in highest rumen undegradable protein fraction in *P. minor*. It indicated that *P. minor* seeds could be used as potential source of by pass protein in comparison to conventional cereal grains. The effective degradability of crude protein of *P. minor* was in between the two cereal grains. The true degradability of crude protein of *P. minor* was the lowest. The *in vitro* and *in sacco* studies exhibited promising results.

Table 4. *In vitro* evaluation of concentrate mixtures containing different level of *Phalaris minor* seeds (irrespective of type of cereal grains)

Parameter	Level of *P.minor* seeds (%)					Pooled SE
	0	25	50	75	100	
NGP (ml/g DM/24 h)	212.9^{c}	209.4^{c}	202.0^{b}	202.5^{b}	195.1^{a}	1.96
NDFD (%)	38.4^{b}	41.8^{b}	40.2^{b}	44.4^{b}	28.9^{a}	2.25
TOMD (%)	76.9^{bc}	77.9^{c}	76.6^{bc}	75.5^{b}	72.9^{a}	0.78
ME (MJ/kg DM)	9.8^{b}	9.3^{a}	9.5^{a}	9.6^{ab}	9.3^{a}	0.11

NGP: Net gas production; NDFD: Neutral detergent fiber digestibility; TOMD: True organic matter digestibility; ME: Metabolisable energy.
Figures with different superscripts in a row differ significantly ($p<0.05$).

Table 5. *In vitro* evaluation of conventional cereal grains based concentrate mixtures (irrespective of the level of *P. minor* seeds)

Parameter	Maize based	Wheat based	Pooled SE
NGP (ml/g DM/24 h)	200.8[a]	207.9[b]	1.24
NDFD (%)	36.9	40.5	1.42
TOMD (%)	75.2[a]	76.8[b]	0.49
ME (MJ/kg DM)	9.3[a]	9.6[b]	0.07

NGP: Net gas production; NDFD: Neutral detergent fiber digestibility; TOMD: True organic matter digestibility; ME: Metabolisable energy.
Figures with different superscripts in a row differ significantly ($p<0.05$).

Replacement of cereal grains with *Phalaris minor* seeds in concentrate mixture

The net gas production was depressed significantly ($p<0.05$) when the cereal grains were replaced with *P. minor* (more than 25 per cent). The net gas production from concentrate mixtures containing 50 and 75 percent *P. minor* was comparable, but higher ($p<0.05$) than that produced from concentrate mixture where cereal grains were completely replaced by *P. minor* (Table 4). The digestibility of OM and NDF was not affected by inclusion of *P. minor,* up to 75 per cent level in concentrate mixture. The availability of ME (MJ/kg DM) from concentrate mixtures containing *P. minor* seeds, varied from 9.3 to 9.6 as compared to control concentrate mixture (9.8). The data conclusively revealed that conventional cereal grains could be replaced without affecting the availability of nutrients, by *P. minor* seeds up to 75 per cent.

On an average, the wheat based concentrate mixture, irrespective of the level of *P. minor,* showed higher net gas production, digestibility of nutrients (NDF and OM) and ME availability as compared to maize based concentrate mixture (Table 5), which supports the *in vitro* and *in sacco* results of the individual cereal grains.

The *in sacco* study revealed that level of *P. minor* showed no significant impact on the rapidly soluble fraction (Table 6). The insoluble but potentially degradable fraction of concentrate mixture containing *P. minor* up to 75 per cent was comparable with that of CCM. The concentrate mixture containing only *P. minor* (100%) had the lowest potentially degradable fraction, but was statistically comparable to that of concentrate mixtures containing 50 and 75 per cent of *P. minor* seeds. The potentially degradable fraction of the concentrate mixtures was degraded at a comparable rate, except that it was significantly depressed at 25 per cent level of replacement. The rumen UDF increased linearly with the increase in level of *P. minor* in the concentrate mixture. However, it was exceptionally low in concentrate mixture where 25 per cent of cereal grains were replaced by *P. minor* seeds. The effective and true degradability of DM of concentrate mixtures containing *P. minor* from 50 to 100 per cent was similar to that of CCM. But, at 25 per cent level of replacement, effective degradability was highest.

The digestion kinetic parameters for CP, irrespective of the type of cereal grains used, in the concentrate mixture showed no consistent trend. The rapidly soluble fraction and insoluble but potentially degradable fraction were comparable in all the groups except at 25 and 50 per cent level of replacement, which differed significantly (Table 6). The degradation rate of CP from the concentrate mixture containing 50 percent *P. minor* was highest but comparable to that of control. The undegradable fraction was observed to be highest in concentrate mixture containing only *P. minor*. The effective degradability of CP of concentrate mixtures containing *P. minor* up to 75 per cent level was statistically comparable to that of CCM but it was depressed ($p<0.05$) at 100 per cent level of replacement.

Irrespective of level of *P. minor*, the digestion kinetic parameters for DM of concentrate mixtures revealed that

Table 6. Digestion kinetic parameters of concentrate mixtures (irrespective of cereal grain used) containing *P. minor* seeds

Parameter	Level of *P.minor* seeds (%)					Pooled SE
	0	25	50	75	100	
Dry matter (%)						
A	46.2	52.0	48.9	50.1	51.8	2.31
B	40.1[b]	39.3[b]	35.2[ab]	33.0[ab]	29.8[a]	2.74
c (h^{-1})	0.09[b]	0.06[a]	0.09[b]	0.09[b]	0.10[b]	0.01
UDF	13.6[b]	8.6[a]	16.0[bc]	16.9[c]	18.4[c]	1.02
ED	77.6[a]	78.6[c]	76.5[a]	76.2[a]	75.6[a]	0.36
TD	78.2[b]	69.5[a]	78.5[b]	78.3[b]	79.5[b]	1.77
Crude protein (%)						
A	55.5[ab]	60.2[b]	51.7[a]	55.6[ab]	51.5[a]	1.65
B	39.7[ab]	36.9[a]	42.4[b]	39.4[ab]	38.9[ab]	1.66
c (h^{-1})	0.086[bc]	0.06[a]	0.096[c]	0.070[a]	0.080[ab]	0.004
UDF	4.8[ab]	2.8[a]	5.9[b]	5.0[ab]	9.5[c]	0.81
ED	86.3[b]	86.6[b]	85.2[b]	84.7[b]	80.9[a]	0.71
TD	77.5[cd]	71.5[a]	79.0[c]	73.5[ab]	75.4[bc]	1.07

a: Rapidly soluble fraction; b: Insoluble but potentially degradable fraction; c: Degradation rate.
ED: Effective degradability; UDF: Undegradable fraction; TD: True degradability.
Figures with different superscripts in a row differ significantly ($p<0.05$).

Table 7. Digestion kinetic parameters of conventional cereal grains based concentrate mixtures (irrespective of level of *P. minor* seeds)

Parameter	Maize based	Wheat based	Pooled SE
Dry matter (%)			
a	46.6[a]	53.0[b]	1.46
b	40.1[b]	30.9[a]	1.74
c (h^{-1})	0.08[a]	0.09[b]	0.01
UDF	13.4[a]	16.1[b]	0.65
ED	76.1[a]	77.6[b]	0.23
TD	74.4[a]	79.2[b]	1.12
Crude protein (%)			
a	56.0	53.8	1.04
b	38.8	40.1	1.05
c (h^{-1})	0.07[a]	0.08[b]	0.003
UDF	5.1	6.1	0.51
ED	84.8	84.7	0.45
TD	73.8[a]	76.9[b]	0.68

a: Rapidly soluble fraction; b: Insoluble but potentially degradable fraction; c: Degradation rate; ED: Effective degradability; UDF: Undegradable fraction; TD: True degradability.
Figures with different superscripts in a row differ significantly ($p<0.05$).

wheat based concentrate mixture had higher ($p<0.05$) rapidly soluble fraction and lower ($p<0.05$) insoluble but potentially degradable fraction as compared to maize based concentrate mixture (Table 7) with no significant effect on the protein fractions. The DM and CP of wheat based concentrate mixture was degraded at a much faster rate ($p<0.05$) as compared to that of maize based concentrate mixture. Wheat based concentrate mixture showed significantly ($p<0.05$) higher true degradability of nutrients (DM and CP). The *in vitro* gas production and *in sacco* studies conclusively revealed that conventional cereal grains (maize and wheat) in the concentrate mixture could be replaced with *P. minor* seeds, up to 75% without any adverse effect on the availability of nutrients.

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